Journal of Medical Virology 56:25–32 (1998)

Attachment of Hepatitis C Virus to Cultured Cells: A Novel Predictive Factor for Successful Interferon Therapy

Yoichi Kimura,[1,2*] Kazuhiro Hayashida,[2] Hiromi Ishibashi,[2] Yoshiyuki Niho,[2] Kouhei Akazawa,[3] and Yusuke Yanagi[1]

[1]*Department of Virology, Faculty of Medicine, Kyushu University, Fukuoka, Japan*
[2]*First Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan*
[3]*Department of Medical Informatics, Kyushu University Hospital, Kyushu University Fukuoka, Japan*

In order to identify reliable predictive factors for the response to interferon (IFN) therapy, we examined 52 patients with chronic hepatitis C virus (HCV) infection who underwent IFN therapy. Titers of free virions that were not complexed with antibodies were determined by immunoprecipitation of the pretreatment sera with antihuman immunoglobulin. Cell attachment titers were determined by inoculating HPB-Ma cells, a human T-cell line, with the patients' pretreatment sera. A sustained remission was achieved in 9 out of 28 patients with genotype 1b infection and in 18 out of 24 patients with genotype 2 infection. The free virion titers and the cell attachment titers correlated well with the serum HCV RNA levels in patients with genotype 1b infection ($r = 0.817$, $P < 0.001$; $r = 0.802$, $P < 0.001$, respectively), but not in those with genotype 2 infection. They exhibited a high correlation with the responsiveness to IFN in all patients combined ($P < 0.001$). A multiple logistic regression analysis was performed to identify the factor most significantly associated with the response to IFN among all confounding factors. The analysis indicated that the cell attachment is the most reliable predictive factor regarding a favorable response to IFN therapy in patients with chronic HCV infection. ***J. Med. Virol. 56:25–32, 1998.***

KEY WORDS: immunoprecipitation; PCR; immune complex

INTRODUCTION

Hepatitis C virus (HCV), a member of the Flaviviridae family, commonly causes posttransfusion and sporadic non-A, non-B hepatitis [Choo et al., 1989; Kuo et al., 1989], and chronic HCV infection can lead to serious liver diseases, including cirrhosis and hepatocellular carcinoma [Kiyosawa et al., 1990]. Currently, interferon (IFN) is the only drug that induces viral clearance and a marked biochemical and histological improvement. However, IFN therapy is expensive, has only an approximately 25–30% response rate [Davis et al., 1989; Di Bisceglie et al., 1989], and can cause significant side effects. For these reasons, it is desirable to predict the efficacy of IFN therapy on HCV infection.

Several factors have been identified that help in predicting the long-term beneficial response of HCV-infected patients to IFN therapy. Previous studies have shown that different HCV strains vary in their responsiveness to IFN therapy and that HCV genotype 2 is more susceptible to IFN than HCV genotype 1b [Hino et al., 1994; Mahaney et al., 1994]. Amino acid sequence variability has also recently been identified in the nonstructural protein 5A (NS5A) of genotype 1b that correlated well with IFN sensitivity [Enomoto et al., 1996]. Furthermore, patients with low pretreatment titers of serum HCV RNA were found to be more susceptible to IFN than those with high HCV RNA levels [Chemello et al., 1995; Garson et al., 1995]. These factors are, however, still insufficient for predicting the response to IFN completely. For instance, there are some cases, especially among patients with genotype 2 infection, that respond to IFN treatment despite high levels of viral RNA [Mita et al., 1994].

HCV virions have been shown to be bound to anti-HCV antibodies as immune complexes in the plasma of patients with chronic HCV infection [Thomssen et al., 1993], and those virions complexed with antibodies have a low infectivity [Hijikata et al., 1993]. Several groups have reported cell culture systems that can support the growth of HCV [Shimizu and Yoshikura, 1994;

Contract grant sponsor: the Ministry of Health and Welfare, Japan.

*Correspondence to: Yoichi Kimura, M.D., Department of Virology, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail: kimuray@intmed1.med.kyushu-u.ac.jp

Accepted 27 March 1998

Kato et al., 1995]. Using a human T-cell line, Shimizu et al. [1993] showed that in vitro infectivity titers of selected strains of HCV paralleled their infectivity titers in chimpanzees. They also developed an in vitro assay for neutralizing antibodies to HCV that bind to virions and prevent the initiation of the replication cycle of HCV in susceptible cells [Shimizu et al., 1994].

In an attempt to identify reliable predictive factors for successful IFN therapy, we examined, in addition to the currently used factors, the amount of free virions and in vitro infectivity in the pretreatment sera of patients with HCV infection who underwent IFN therapy. Our results showed viral attachment to cultured cells to be the most reliable predictive factor for the response to IFN therapy.

MATERIALS AND METHODS

Patients

We studied 52 patients with chronic HCV infection who underwent IFN therapy between January 1995 and December 1996. All patients had elevated serum aminotransferase levels for at least 6 months and had biopsy-proven chronic hepatitis. They were positive for serum anti-HCV antibodies (the second-generation assay) and serum HCV RNA, and showed no evidence of hepatitis B virus infection, or alcoholic, autoimmune, or any other type of liver disease. After informed consent had been obtained from each patient, a total of 800 million units of recombinant IFN-α 2b (Intron A, Schering-Plough, Kenilworth, NJ) was administered intramuscularly for 24 weeks (by daily injection during the first 2 weeks and 3 times per week thereafter). Each month during and after the therapy, patients were seen and examined for biochemical and virological tests. All patients were followed for at least 6 months after the completion of the therapy and were assigned to one of the three groups according to the outcome of treatment. Complete responders to IFN therapy were defined as those with absence of serum HCV RNA by nested reverse transcriptase-polymerase chain reaction (RT-PCR) both during the therapy and 6 months after completion of the therapy. Nonresponders had detectable HCV RNA in serum by RT-PCR at the end of the treatment. Transient responders showed an initial reduction of HCV RNA with subsequent relapse within 6 months after the completion of the therapy. Six months after the completion of IFN therapy, 27 patients showed a sustained response to IFN treatment (complete responders), and 25 patients did not respond to IFN treatment (nonresponders). Nonresponders include 13 transient responders. Clinical and virological profiles of the patients studied are summarized in Table I. Pretreatment sera were collected on the starting day of IFN therapy and stored at −80°C until use.

TABLE I. Clinical Profile of 52 Patients With Chronic Hepatitis C Before IFN Therapy

	Complete responders	Nonresponders
Number of patients	27	25
Age (year)		
<50	17	12
⩾50	10	13
Sex (M/F)	23/4	19/6
Blood transfusion (Y/N)	5/22	4/21
Serum ALT[a] (IU/L)		
<100	15	19
⩾100	12	6
Liver histology[b]		
CPH	2	4
CAH2A	22	18
CAH2B	3	3
Serum HCV RNA[c] (copies/50 μl)		
$\leqslant 10^{5.5}$	23	12
$\geqslant 10^{5.5}$	4	13
Genotype (1b/2)[c]	9/18	19/6

[a]ALT = alanine aminotransferase. Normal range, to 38 IU per liter.
[b]CPH = chronic persistant hepatitis. CAH = chronic active hepatitis. European classification.
[c]$P < 0.01$ (Fisher's exact test).

Blood Testing

Serum HCV RNA levels were determined by a competitive RT-PCR assay [Kato et al., 1993]. The HCV genotype was determined by RT-PCR with type-specific primers according to the method of Okamoto et al. [1992]. Determination of the nucleotide and deduced amino acid sequences of the IFN sensitivity determining region was performed for patients with HCV-1b infection as described previously [Enomoto et al., 1996].

Immunoprecipitation

HCV RNA in circulating immune complexes was detected as described previously with a slight modification [Hijikata et al., 1993]. Ten μl of serum was mixed with 100 μl of an undiluted IgG fraction of goat anti-human immunoglobulins (IgA + IgG + IgM, Organon Teknika, NC). The mixture was incubated overnight at 4°C, and then separated into supernatant and pellet fractions by centrifugation at 680 × g for 15 min. The pellet fractions were rinsed once with phosphate-buffered saline. Nucleic acids were extracted from each fractions using a nucleotide extraction kit (SMITEST, Sumitomo, Japan) and then serially diluted as shown in Figure 1A. An aliquot from each dilution was tested by nested RT-PCR for the presence of HCV RNA [Ito et al., 1996]. The primers used were from the 5′ noncoding region of the HCV genome [Nakatsuji et al., 1992]. The first sense primer was 5′-ACTCCACCATAGATCACTCC-3′; the first antisense primer was 5′-AACACTACTCGGCTAGCAGT-3′. The second sense primer was 5′-TTCACGCAGAAAGCGTCTAG-3′; the second antisense primer 5′-GTTTATCCAAGAAAGGACCC-3′. The amplified DNA was visualized by agarose gel electrophoresis (2%) and ethidium bromide staining. The free virion titer was determined from the maximum dilution of the supernatant nucleic acids in

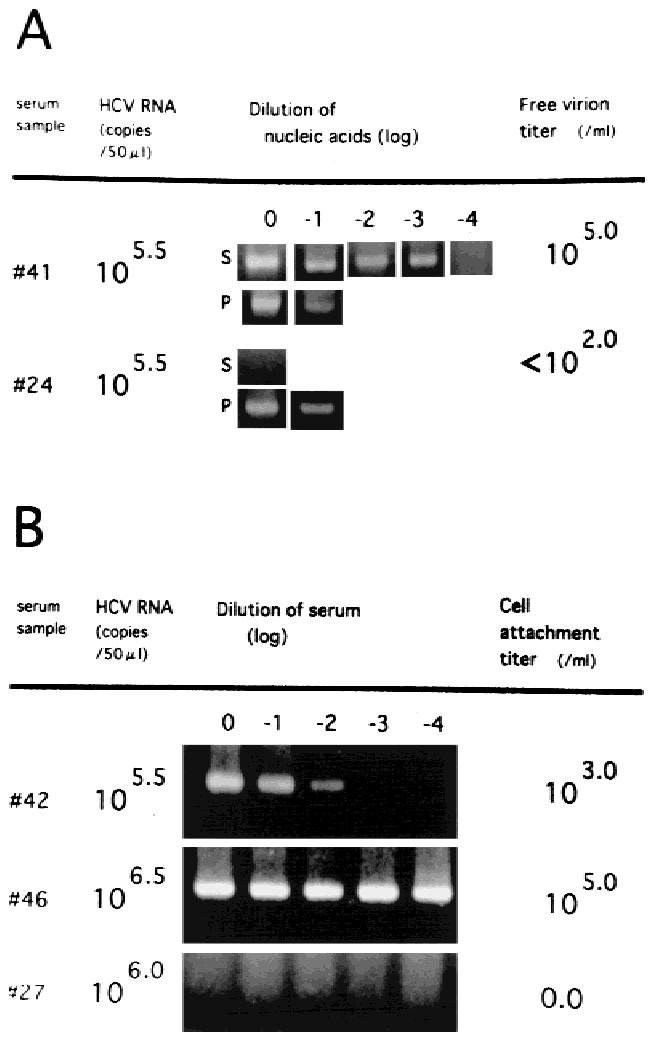

Fig. 1. Agarose gel electrophoresis of the PCR products for detecting HCV RNAs. **A:** RNAs in the supernatant and pellet fractions after immunoprecipitation of pretreatment sera (cases 41 and 24 are shown as examples). S denotes supernatant; P, pellet. The serum HCV RNA levels and free virion titers are also shown. **B:** RNAs from the HPB-Ma cells inoculated with serially diluted pretreatment sera 42, 46, and 27. The serum HCV RNA levels and cell attachment titers are also shown.

which HCV RNA could be detected and was expressed as values/ml.

HCV Attachment to Cultured Cells

HPB-Ma cells, a human T-cell line, have been shown to support the replication of HCV efficiently [Shimizu et al., 1993]. HPB-Ma cells (1×10^5 cells in 100 μl) were inoculated with 100 μl of undiluted or 10^{-1}, 10^{-2}, 10^{-3}, or 10^{-4} diluted solution of pretreatment sera. After adsorption at 37°C for 2 hr, the cells were washed three times. The total RNA was extracted from the cells, and nested RT-PCR was performed to detect HCV RNA. When HCV RNA was not detected in the cells inoculated with a nondiluted sample, we defined the titer to be zero. The titer of the attachment was the average of two independent measurements and was expressed as values/ml.

Statistics

In the univariate analysis, the Fisher's exact test was performed to assess the significance of differences in proportions. The Spearman rank correlation coefficient was used to estimate the magnitude of the correlation between the free virion titer and the serum HCV RNA level. In addition, the following factors were analyzed by the multivariate logistic regression analysis with stepwise regression method to determine which factor was most significantly related to the efficacy of IFN: age (<50, ⩾50), sex, history of transfusion, pretreatment level of serum alanine aminotransferase (<100, ⩾100), liver histology on biopsy (chronic persistent hepatitis, chronic active hepatitis 2A, chronic active hepatitis 2B; European classification), level of serum HCV RNA (<5.5, ⩾5.5), HCV genotype (1b, 2), free virion titer (⩽3.0, >3.0), and cell attachment titer (=0.0, >0.0). The multiple logistic regression analysis was performed using overall patients. All analyses were done with the BMDP statistical software (BMDP, Los Angels, CA) and Stat Xact 3 (CYTEL Software, Cambridge, MA). Statistical significance was defined as $P < 0.05$.

RESULTS

Clinical and Virological Data in Patients

Clinical and virological data of 52 patients examined in this study are shown in Table I. The amount of HCV RNA was significantly larger in nonresponders ($P < 0.01$), and there was a significant difference in genotypes between complete responders and nonresponders ($P < 0.01$). These findings are consistent with previous studies [Hino et al., 1994; Mahaney et al., 1994; Chemello et al., 1995; Garson et al., 1995].

Free Virion Titer and Response to IFN

To determine the levels of the immune complex formation between HCV and anti-HCV antibodies in the pretreatment sera of patients with HCV infection, we performed immunoprecipitation using antihuman immunoglobulin. The sera were separated into a supernatant fraction containing free virions and a pellet fraction that was precipitated with antihuman immunoglobulin and contained virus–antibody complexes. Nucleic acids were isolated from each fraction and subjected to the RT-PCR assay for detecting HC RNA after 10-fold serial dilutions. In order to determine the titer of free virions, nucleic acids from the supernatant fractions were diluted down to 10^{-4}. Figure 1A shows two examples of this analysis. In serum 41, HCV RNA was detected in 10^0 (undiluted), 10^{-1}, 10^{-2}, and 10^{-3} diluted supernatant samples as well as in 10^0 and 10^{-1} diluted pellet samples. In serum 24, HCV RNA was detected in the undiluted and 10^{-1} diluted pellet samples, but not in the supernatant fraction.

The results of this analysis for all the patients examined are shown in Table II. The patients were divided into two groups according to the response to IFN therapy, and the genotype of HCV was also indicated.

TABLE II. Titers of Free Virions and Cell Attachment in the Pretreatment Sera of Patients With HCV Infection[a]

Serum number	genotype	IFN response	HCV-RNA (log copies/50μl)	NS5A[b]	Free virion titer (log/ml)	Attachment titer (log/ml)
1	1b	CR	2.0	4	<2	0
2	1b	CR	2.5	5	<2	0
3	1b	CR	3.0	5	2	0
4	1b	CR	3.5	8	2	0
5	1b	CR	3.5	4	3	0
6	1b	CR	3.5	1	<2	0
7	1b	CR	4.0	4	3	0
8	1b	CR	4.5	7	4	0
9	1b	CR	5.0	1	3	1.5
10	2a	CR	2.5	n	<2	0
11	2a	CR	3.0	n	3	0
12	2a	CR	4.0	n	<2	0
13	2a	CR	4.0	n	<2	0
14	2a	CR	4.0	n	<2	0
15	2b	CR	4.5	n	<2	0
16	2b	CR	4.5	n	<2	0
17	2b	CR	4.5	n	2	0.5
18	2a	CR	5.0	n	<2	0
19	2a	CR	5.0	n	<2	0
20	2b	CR	5.0	n	3	0
21	2a	CR	5.0	n	<2	0
22	2a	CR	5.0	n	2	0
23	2b	CR	5.0	n	4	2.0
*24	2a	CR	5.5	n	<2	0
25	2a	CR	5.5	n	<2	0
26	2a	CR	5.5	n	3	0
*27	2b	CR	6.0	n	<2	0
28	1b	NR(TR)	3.5	0	2	2.0
29	1b	NR(TR)	4.0	2	4	1.0
30	1b	NR(TR)	4.0	1	3	1.5
31	1b	NR	4.5	2	4	3.5
32	1b	NR(TR)	4.5	0	3	1.0
33	1b	NR(TR)	4.5	5	4	3.0
34	1b	NR	5.0	0	4	2.0
35	1b	NR	5.0	0	3	4.0
36	1b	NR	5.0	1	4	3.5
37	1b	NR	5.5	0	3	2.5
38	1b	NR	5.5	0	3	2.5
39	1b	NR	5.5	1	4	3.5
40	1b	NR(TR)	5.5	0	4	2.0
*41	1b	NR(TR)	5.5	1	5	3.5
*42	1b	NR	5.5	0	4	3.0
43	1b	NR(TR)	5.5	2	4	3.5
44	1b	NR(TR)	6.0	0	5	2.5
45	1b	NR	6.0	0	5	4.0
*46	1b	NR	6.5	0	4	5.0
47	2a	NR(TR)	5.0	n	3	0.5
48	2a	NR(TR)	5.0	n	3	2.5
49	2b	NR(TR)	5.0	n	4	2.5
50	2b	NR(TR)	5.5	n	3	2.5
51	2a	NR	6.0	n	5	2.5
52	2b	NR	6.5	n	2	1.0

[a]CR denotes complete responder; NR, nonresponder; TR, transient responder; n, not tested. Asterisk denotes that electrophoresis with the indicated serum was shown in Figure 1.
[b]The number of amino acid changes in NS5A, compared with the prototype sequence of genotype 1b.

The majority of patients, regardless of the eventual response to IFN, had HCV–anti-HCV antibody complexes, as indicated by the presence of HCV RNA in the pellet fraction (data not shown).

We examined the relationship between the free virion titer and the serum HCV RNA. Results showed that the free virion titers directly correlated with the serum HCV RNA levels in the genotype 1b ($r = 0.817$, $P < 0.001$) (Fig. 2A). In contrast, no significant relationship was observed between the free virion titers and the serum HCV RNA levels in the genotype 2 ($r = 0.260$, $P = 0.223$) (Fig. 2B). Figure 2 also showed complete responders to have lower free virion titers than nonresponders.

Cell Attachment Titer and Response to IFN

The immunoprecipitation analysis described above only detects HCV RNA and did not take the infectivity of virus particles into account. Thus, free virion may not be infectious because of mutations or particle deg-

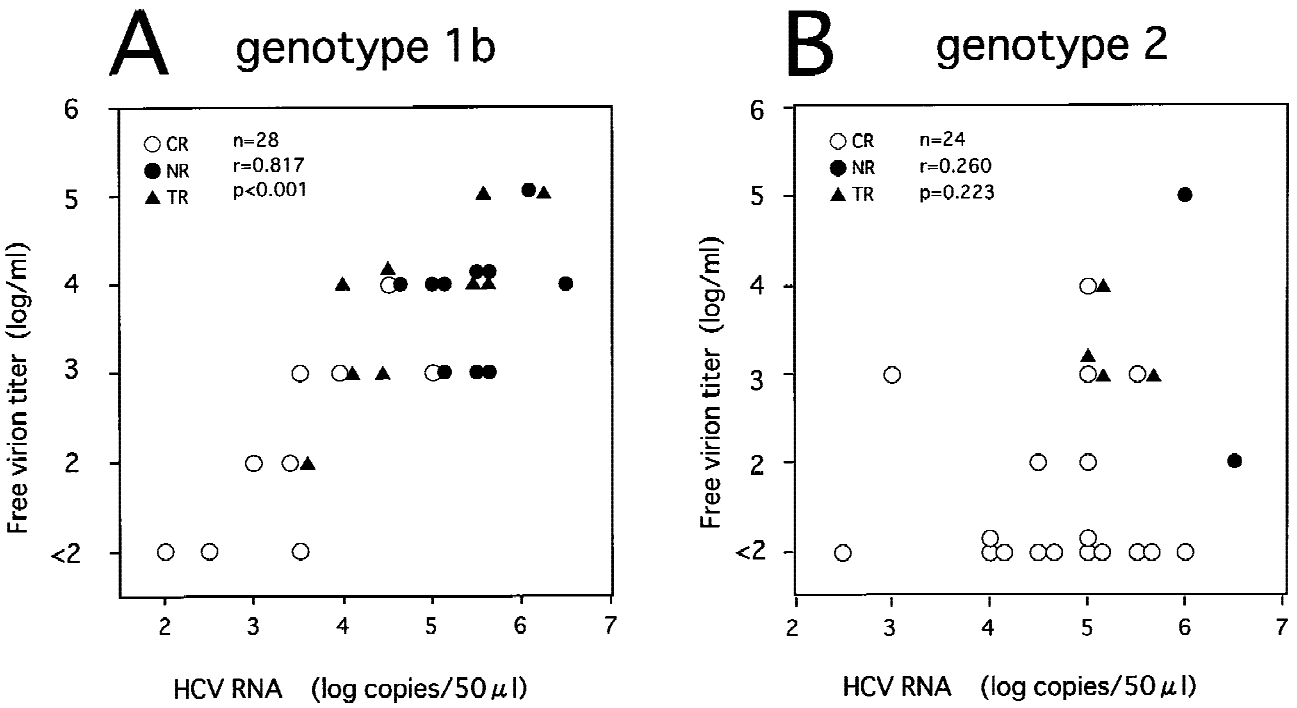

Fig. 2. Correlation between the serum HCV RNA level and the free virion titer in patients with genotype 1b **(A)** and with genotype 2 **(B).** CR denotes complete responder; NR, nonresponder; TR, transient responder.

radation. On the other hand, immune complexes may still be infectious if the virions are not complexed with neutralizing antibodies. We therefore examined the cell attachment of HCV in the patients' sera. Pretreatment sera were serially diluted in 10-fold increments, and 100 μl of each dilution was mixed with HPB-Ma cells. After incubation at 37°C for 2 hr, the cells were washed extensively, and then the virus particles that bound and penetrated into the cells were detected by RT-PCR (Fig. 1B). Serum 42 gave the positive signal in the 10^{-2} but not in the 10^{-3} diluted sample. The cell attachment titer per ml of this serum was 10^3/ml. Similarly, the cell attachment titers of serums 46 and 27 were determined to be 10^5 and 0.0/ml, respectively. The results in Table II showed the cell attachment titer of pretreatment sera to be zero in 8 out of 9 complete responders of genotype 1b and in 16 out of 18 complete responders of genotype 2. In contrast, all of the nonresponders showed high cell attachment titers, ranging from $10^{0.5}$ to $10^{5.0}$/ml.

The correlation between the cell attachment titer and the serum HCV RNA was examined. The results in Figure 3 demonstrated the cell attachment titers to correlate directly with the HCV RNA levels in genotype 1b ($r = 0.802$, $P < 0.001$), but not in genotype 2 ($r = 0.344$, $P = 0.100$). Furthermore, Figure 3 also showed that all patients with the cell attachment titer of less than $10^{0.5}$/ml exhibited a complete response to IFN. None of the patients with the titer of more than $10^{2.0}$/ml showed a response to IFN, and only 28% of those with the titer of $10^{0.5}$ to $10^{2.0}$/ml responded to IFN.

Correlation With IFN Responsiveness

The predictive values of various factors were assessed by examining their correlation with the response to IFN therapy. When all the patients were combined regardless of the genotype, both the free virion titer and the cell attachment titer highly correlated with the response to IFN ($P < 0.001$) (Fig. 4). In addition to these factors, the number of amino acid changes in NS5A, compared to the wild-type sequence, also showed a good correlation with the response to IFN in patients with HCV 1b infection (Table III), thus confirming previously reported results [Enomoto et al., 1996].

To identify the factor most significantly associated with the response to IFN, a multiple logistic regression analysis was carried out as described in Materials and Methods and infinite estimate for the regression coefficient of the cell attachment titer was obtained. This result indicated almost a complete correlation between the cell attachment titer and the IFN response.

DISCUSSION

Other groups have shown that the levels of circulating viral RNA and the genotype of HCV are the major determinants of the response to IFN therapy in patients with chronic HCV infection [Mahaney et al., 1994; Chemello et al., 1995]. Recently, Enomoto et al. [1996] reported that mutations in nonstructural protein 5A gene also influence the response in patients with genotype 1b infection. Although these factors have proven to be useful in predicting the outcomes of

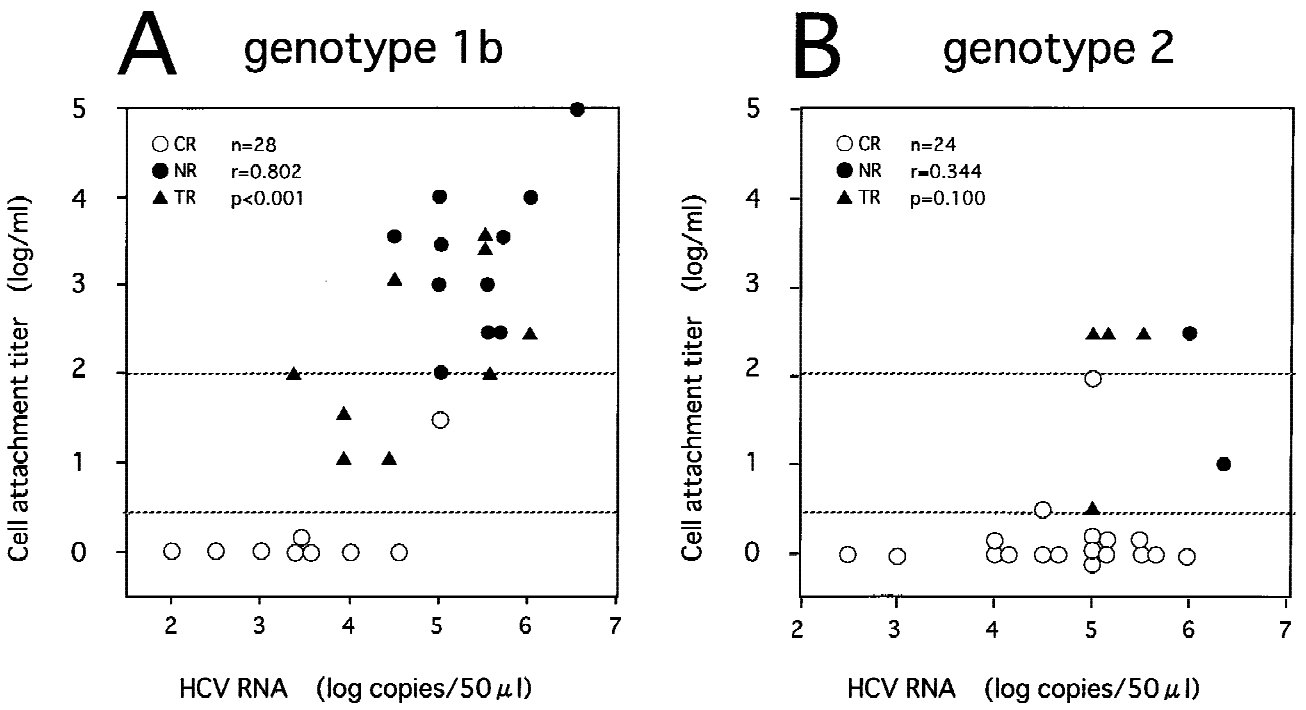

Fig. 3. Correlation between the serum HCV RNA level and the cell attachment titer in genotype 1b **(A)** and genotype 2 **(B).** CR denotes complete responder; NR, nonresponder; TR, transient responder.

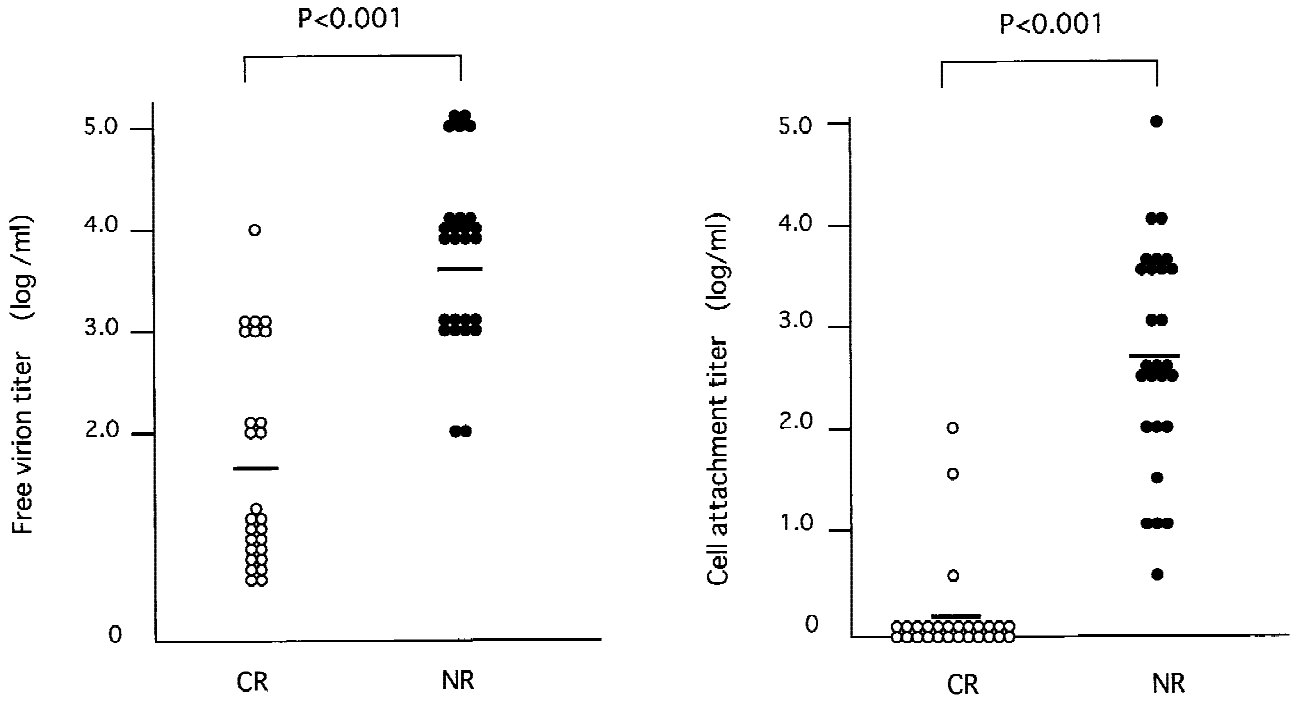

Fig. 4. Comparisons of the free virion titer and the cell attachment titer between complete responders (CR) and nonresponders (NR).

IFN therapy, there have been many cases in which they failed to make an accurate prediction. Furthermore, information on the sequence of NS5A is applicable only to patients with genotype 1b infection. In this article, we evaluated other potential predictive factors regarding the response to IFN.

It has been shown that HCV is complexed with antibodies in the patients' sera [Hijikata et al., 1993; Thomssen et al., 1993], and that virus–antibody complexes have a low infectivity [Farci et al., 1994; Shimizu et al., 1994]. We measured the titer of free virions that were not bound to antibodies in the pre-

TABLE III. Correlation of Free Virion Titer, Cell Attachment Titer, and Amino Acid Changes in NS5A With Response to Interferon in Patients With Genotype 1b Infection

IFN response	Complete responder	Nonresponder	*P* value[a]
Free virion titer (log/ml)			
≤3.0	8	6	<0.05
>3.0	1	13	
Cell attachment titer (log/ml)			
=0.0	8	0	<0.001
>0.0	1	19	
Amino acid changes in NS5A[b]			
0	0	11	<0.001
1–3	2	7	
≥4	7	1	

[a]Fisher's exact test.
[b]Compared with the prototype sequence of genotype 1b.

treatment sera of patients, reasoning that it may better reflect the true infectivity of virus than the level of circulating viral RNA. We also determined viral attachment to cultured cells, using a recently described cell culture system. Our results showed these factors to be useful for predicting the outcome of IFN therapy.

To identify the factors associated with the response to IFN, a multivariate analysis should be used for a simultaneous multivariate adjustment of all confounding factors. We performed a multiple logistic regression analysis and obtained the infinite estimate for the regression coefficient of the cell attachment titer. This phenomenon was caused by monotone likelihood [Bryson and Johnson, 1981], which indicates almost a complete correlation between the cell attachment titer and the IFN response. Our results thus indicated the cell attachment titer to be the most powerful predictor for the response to IFN among all the predictors examined.

Using a human T-cell line HPB-Ma, Shimizu et al. [1994] showed that HCV replication as measured by the detection of HCV RNA within the cells for several days after infection was comparable to the titer of the virus that bound and penetrated into cells in the first 2 hr of incubation. They also demonstrated the in vitro infectivity titers of selected strains of HCV to parallel their infectivity titers in chimpanzees [Shimizu et al., 1993]. Thus, unlike the measurement of circulating viral RNA or free virions, the cell attachment studied in this article appears to have more biological relevance. This may explain the discrepancies between the cell attachment titer and the free virion titer in some cases, as observed in Table II. Those cases that had high titers of free virion, but low cell attachment titers, presumably contained HCV mutants that could not bind to cells or penetrate into cells efficiently, or, more likely, the damaged HCV virions and exposed nucleocapsids that resulted from antibody binding to intact and infectious virions.

Patients with genotype 2 infection showed significantly lower titers of free virion (Fig. 2) and cell attachment (Fig. 3), compared to those with genotype 1b infection. This may explain the previous findings that genotype 2 is more susceptible to IFN than genotype 1b. While free virion titers correlated well with HCV RNA in patients with genotype 1b, there were many genotype 2 cases that had high levels of HCV RNA but low titers of free virion. The majority of virions in these patients thus seem to be complexed with antibodies. The mechanism by which genotype 2 virions form more immune complexes than genotype 1b virions has yet to be defined. The antibody responses toward specific HCV proteins have been shown to differ between genotypes [Yuki et al., 1995]. Furthermore, the antibody responses to the hypervariable region in the N-terminus of the E2 protein [Weiner et al., 1991], a neutralizing epitope [Zibert et al., 1995], were higher in patients with genotype 2a than in those with genotype 1b [Yoshioka et al., 1997]. These data suggest that the difference in immune complex formation is, at least partly, due to different humoral immune responses between genotypes.

The mechanism by which IFN cures patients with HCV infection is not known, but IFN presumably inhibits the replication of HCV within cells. The fact that the low cell attachment titer is the best indicator for the response to IFN may thus suggest that in order for IFN to be effective, antibodies and probably cytotoxic T-cells first have to reduce the titer of infectious HCV.

The measurement of HCV attachment to cultured cells is biologically relevant and easily performed. Thus, it should be used to predict the response of patients with HCV to IFN.

ACKNOWLEDGMENTS

We thank Dr. Yohko K. Shimizu for providing us with the cell line HPB-Ma and the control plasmas, as well as for her helpful suggestions. We also thank Dr. Minako Hijikata for her technical suggestions.

REFERENCES

Bryson MC, Johnson ME (1981): The incidence of monotone likelihood in the Cox model. Technometrics 23:381–383.

Chemello L, Bonetti P, Cavalletto L, Talato F, Donadon V, Casarin P, Belussi F, Frezza M, Noventa F, Pontisso P, Benvegnu L, Casarin C, Alberti A, the TriVeneeto Viral Hepatitis Group (1995): Randomized trial comparing three different regimens of alpha-2a-interferon in chronic hepatitis C. Hepatology 22:700–706.

Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M (1989): Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 244:359–362.

Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC, Jr., Perrillo RP, Carey W, Jacobson IM, Payne J, Dienstag JL, Vanthiel DH, Tamburro C, Lefkowitch J, Albrecht J, Meschievitz C, Ortego TJ, Gibas A, the Hepatitis Interventional Therapy Group (1989): Treatment of chronic hepatitis C with recombinant interferon-alpha: A multicenter randomized, controlled trial. New England Journal of Medicine 321:1501–1506.

Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, Goodman Z, Banks SM, Hoofnagle JH (1989): Recombinant interferon-alpha therapy for chronic hepatitis C: A randomized, double-blind, placebo-controlled trial. New England Journal of Medicine 321:1506–1510.

Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C (1996): Mutations in

the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. New England Journal of Medicine 334:77–81.

Farci P, Alter HJ, Wong DC, Miller RH, Govindarajan S, Engle R, Shapiro M, Purcell RH (1994): Prevention of hepatitis C virus infection in chimpanzees after antibody-mediated in vitro neutralization. Proceedings of the National Academy of Sciences U.S.A. 91:7792–7796.

Garson JA, Brillanti S, Whitby K, Foli M, Deaville R, Masci C, Miglioli M, Barbara L (1995): Analysis of clinical and virological factors associated with response to alpha interferon therapy in chronic hepatitis C. Journal of Medical Virology 45:348–353.

Hijikata M, Shimizu YK, Kato H, Iwamoto A, Shih JW, Alter HJ, Purcell RH, Yoshikura H (1993): Equilibrium centrifugation studies of hepatitis C virus: Evidence for circulating immune complexes. Journal of Virology 67:1953–1958.

Hino K, Sainokami S, Shimoda K, Iino S, Wang Y, Okamoto H, Miyakawa Y, Mayumi M (1994): Genotypes and titers of hepatitis C virus for predicting response to interferon in patients with chronic hepatitis C. Journal of Medical Virology 42:299–305.

Ito T, Mukaigawa J, Zuo J, Hirabayashi Y, Mitamura K, Yasui K (1996): Cultivation of hepatitis C virus in primary hepatocyte culture from patients with chronic hepatitis C results in release of high-titer infectious virus. Journal of General Virology 77:1043–1054.

Kato N, Yokosuka O, Hosoda K, Ito Y, Ohto M, Omata M (1993): Quantification of hepatitis C virus by competitive reverse transcription-polymerase chain reaction: Increase of the virus in advanced liver disease. Hepatology 18:16–20.

Kato N, Nakazawa T, Mizutani T, Shimotohno K (1995): Susceptibility of human T-lymphotropic virus type I-infected cell line MT-2 to hepatitis C virus infection. Biochemical & Biophysical Research Communications 206:863–869.

Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Furuta S, Akahane Y, Nishioka K, Purcell RH, Alter HJ (1990): Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: Analysis by detection of antibody to hepatitis C virus. Hepatology 12:671–675.

Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE, Tegtmeier GE, Bonino F, Colombo M, Lee WS, Kuo C, Berger K, Shuster JR, Overby LR, Bradley DW, Houghton M (1989): An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 244:362–364.

Mahaney K, Tedeschi V, Maertens G, Di Bisceglie AM, Vergalla J, Hoofnagle JH, Sallie R (1994): Genotypic analysis of hepatitis C virus in American patients. Hepatology 20:1405–1411.

Mita E, Hayashi N, Hagiwara H, Ueda K, Kanazawa Y, Kashara A, Fusamoto H, Kamada T (1994): Predicting interferon therapy efficacy from hepatitis C virus genotype and RNA titer. Digestive Diseases & Sciences 39:977–982.

Nakatsuji Y, Matsumoto A, Tanaka E, Ogata H, Kioysawa K (1992): Detection of chronic hepatitis C virus infection by four diagnostic systems: First-generation and second-generation enzyme-linked immunosorbent assay, second-generation recombinant immunoblot assay and nested polymerase chain reaction analysis. Hepatology 16:300–305.

Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y, Tanaka T, Sato K, Tsuda F, Miyakawa Y, Mayumi M (1992): Typing hepatitis C virus by polymerase chain reaction with type-specific primers: Application to clinical surveys and tracing infectious sources. Journal of General Virology 73:673–679.

Shimizu YK, Purcell RH, Yoshikura H (1993): Correlation between the infectivity of hepatitis C virus in vivo and its infectivity in vitro. Proceedings of the National Academy of Sciences U.S.A. 90: 6037–6041.

Shimizu YK, Yoshikura H (1994): Multicycle infection of hepatitis C virus in cell culture and inhibition by alpha and beta interferons. Journal of Virology 68:8406–8408.

Shimizu YK, Hijikata M, Iwamoto A, Alter HJ, Purcell RH, Yoshikura H (1994): Neutralizing antibodies against hepatitis C virus and the emergence of neutralization escape mutant viruses. Journal of Virology 68:1494–1500.

Thomssen R, Bonk S, Thiele A (1993): Density heterogeneities of hepatitis C virus in human sera due to the binding of b-lipoproteins and immunoglobulins. Medical Microbiology and Immunology 182:329–334.

Weiner AJ, Brauer MJ, Rosenblatt J, Richman KH, Tung J, Crawford K, Bonino F, Saracco G, Choo QL, Houghton M, Han JH (1991): Variable and hypervariable domains are found in the regions of HCV corresponding to the flavivirus envelope and NS1 proteins and the pestivirus envelope glycoproteins. Virology 180:842–848.

Yoshioka K, Aiyama T, Okumura A, Takayanagi M, Iwata K, Ishikawa T, Nagai Y, Kakumu S (1997): Humoral immune response to the hypervariable region of hepatitis C virus differs between genotypes 1b and 2a. Journal of Infectious Diseases 175:505–510.

Yuki N, Hayashi N, Mita E, Hagiwara H, Oshita M, Ohkawa K, Katayama K, Kasahara A, Fusamoto H, Kamada T (1995): Clinical characteristics and antibody profiles of chronic hepatitis C patients: Relation to hepatitis C virus genotypes. Journal of Medical Virology 45:162–167.

Zibert A, Schreier E, Roggendorf M (1995): Antibodies in human sera specific to hypervariable region 1 of hepatitis C virus can block viral attachment. Virology 208:653–661.